† ***Electronic Supplementary Information***

G-quadruplex-binding ligand-induced DNA synapsis inside a DNA origami frame†

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†Electronic supplementary information (ESI) available: DNA sequences used, additional AFM images and real-time HS-AFM movies. See DOI:

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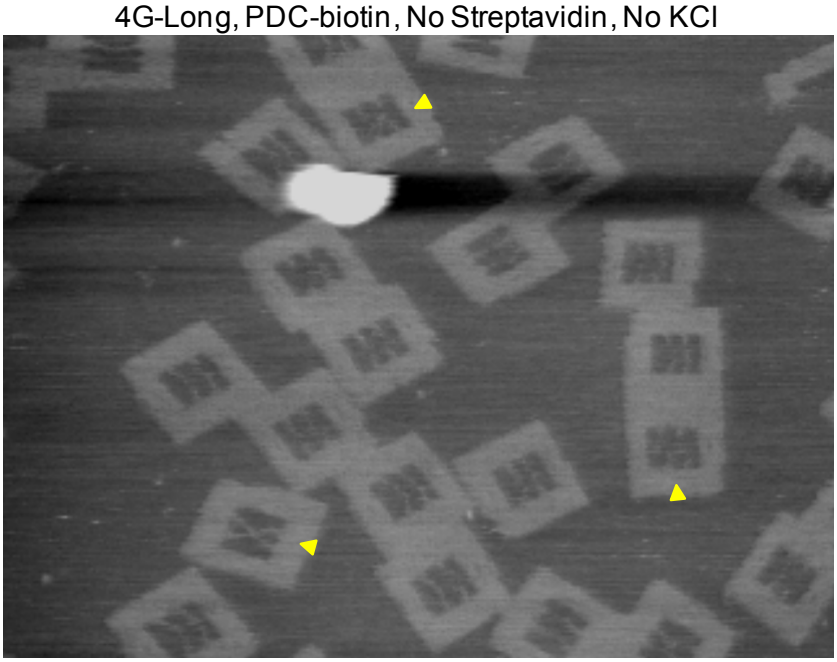
Note on the supplementary movies: The image acquisition speed was 0.2 frame/s. However, the frame rate in the movies is 5 times speed enhanced (1 frame/s). Image acquisition time is given at the top left corner in each frame. Image size: 125 × 125 nm.

Table S1 The list of DNA sequences used in this study.

Name	Sequences (from 5′ to 3′)
Top 3G – 99 nts	CTGTAGCTCATCATGTGGAGACTCTAGAGTGTTCCTGATGGCCGTGAAGGGTTCAAGGCGGTGGGTGCGCGTTGCTCCTCACTGAACACCCTGAACAAA
Top 3G – 67 nts	AGTGAGGAGCAACGCGCACCCACCGCCTTGAAGGGTTCACGGCCATCAGGAACACTCTAGAGTCTCC
Bottom 3G – 109 nts	CGACAATAAACAACATGAGGAAGTGAGGAGCAACGCGCACCCACCGCCTTGAAGGGTTCACGGCCATCAGGAACACTCTAGAGTCTCCGCTCGAGCAAACAAGAGAATC
Bottom 3G – 77 nts	CGAGCGGAGACTCTAGAGTGTTCCTGATGGCCGTGAAGGGTTCAAGGCGGTGGGTGCGCGTTGCTCCTCACTTCCTC
Bottom 3G – 99 nts	CGACAATAAACAACATAGTGAGGAGCAACGCGCACCCACCGCCTTGAAGGGTTCACGGCCATCAGGAACACTCTAGAGTCTCCAGCAAACAAGAGAATC
Bottom 3G – 67 nts	GGAGACTCTAGAGTGTTCCTGATGGCCGTGAAGGGTTCAAGGCGGTGGGTGCGCGTTGCTCCTCACT
Top 4G – 99 nts	CTGTAGCTCATCATGTGGAGACTCTAGAGTGTTCCTGATGGCCGTGAAGGGGTCAAGGCGGTGGGTGCGCGTTGCTCCTCACTGAACACCCTGAACAAA
Top 4G – 67 nts	AGTGAGGAGCAACGCGCACCCACCGCCTTGAGGGGTTCACGGCCATCAGGAACACTCTAGAGTCTCC
Bottom 4G – 109 nts	CGACAATAAACAACATGAGGAAGTGAGGAGCAACGCGCACCCACCGCCTTGAAGGGGTCACGGCCATCAGGAACACTCTAGAGTCTCCGCTCGAGCAAACAAGAGAATC
Bottom 4G – 77 nts	CGAGCGGAGACTCTAGAGTGTTCCTGATGGCCGTGAGGGGTTCAAGGCGGTGGGTGCGCGTTGCTCCTCACTTCCTC
Bottom 4G – 99 nts	CGACAATAAACAACATAGTGAGGAGCAACGCGCACCCACCGCCTTGAAGGGGTCACGGCCATCAGGAACACTCTAGAGTCTCCAGCAAACAAGAGAATC
Bottom 4G – 67 nts	GGAGACTCTAGAGTGTTCCTGATGGCCGTGAGGGGTTCAAGGCGGTGGGTGCGCGTTGCTCCTCACT
Top 6G – 99 nts	CTGTAGCTCATCATGTGGAGACTCTAGAGTGTTCCTGATGGCCGTAGGGGGGTCAAGGCGGTGGGTGCGCGTTGCTCCTCACTGAACACCCTGAACAAA
Top 6G – 67 nts	AGTGAGGAGCAACGCGCACCCACCGCCTTGAGGGGGGTACGGCCATCAGGAACACTCTAGAGTCTCC
Bottom 6G – 109 nts	CGACAATAAACAACATGAGGAAGTGAGGAGCAACGCGCACCCACCGCCTTAGGGGGGTCACGGCCATCAGGAACACTCTAGAGTCTCCGCTCGAGCAAACAAGAGAATC
Bottom 6G – 77 nts	CGAGCGGAGACTCTAGAGTGTTCCTGATGGCCGTGAGGGGGGTAAGGCGGTGGGTGCGCGTTGCTCCTCACTTCCTC
Bottom 6G – 99 nts	CGACAATAAACAACATAGTGAGGAGCAACGCGCACCCACCGCCTTAGGGGGGTCACGGCCATCAGGAACACTCTAGAGTCTCCAGCAAACAAGAGAATC
Bottom 6G – 67 nts	GGAGACTCTAGAGTGTTCCTGATGGCCGTGAGGGGGGTAAGGCGGTGGGTGCGCGTTGCTCCTCACT

a



b

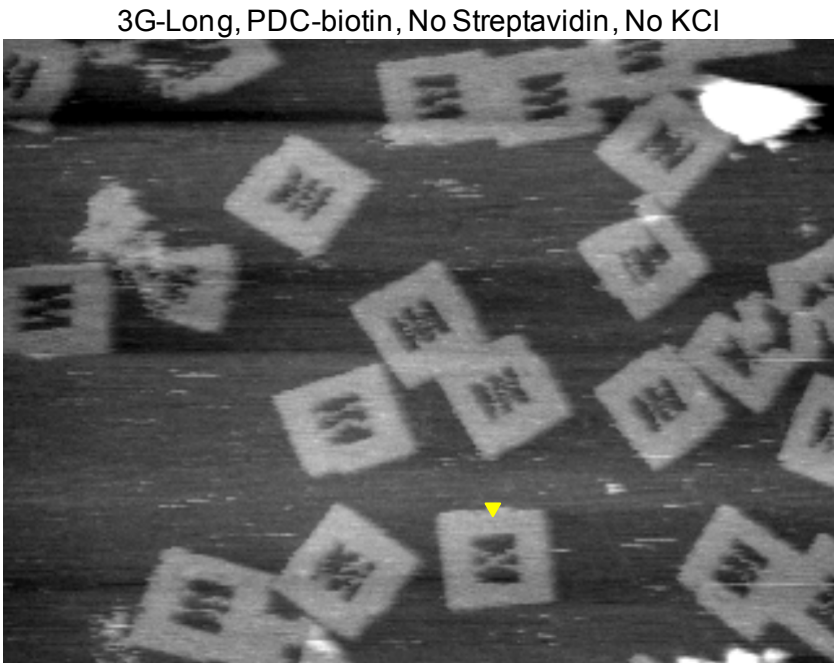
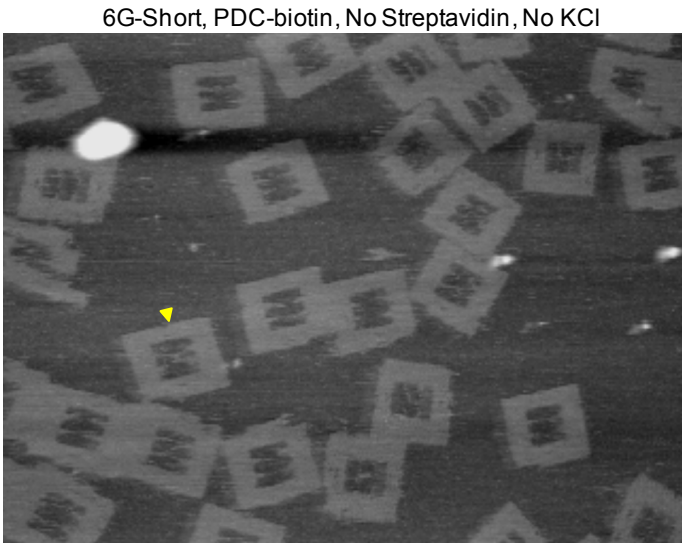


Fig. S1 Zoom-out images of the origami assembly with long-duplexes (67-mer top and 77-mer bottom duplexes) containing four (a) and three (b) contiguous guanines and in the presence of PDC-biotin alone. Yellow arrow heads indicate the X-shapes. [Tris-HCl] = 20 mM, pH 7.6; [$MgCl_2$] = 10 mM; [EDTA] = 1 mM; [PDC-biotin] = 1 μM. Image size: 800 × 600 nm.

a



b

c

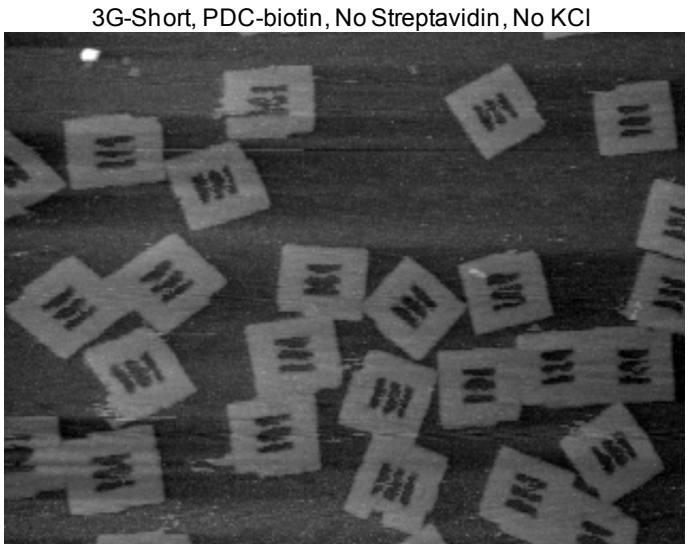


Fig. S2 Zoom-out AFM images of the origami assembly with short-duplexes (both top and bottom duplexes are 67-mer) containing six (a), four (b) and three (c) contiguous guanines and in the presence of PDC-biotin alone. Yellow arrow head indicates the X-shape. [Tris-HCl] = 20 mM, pH 7.6; [$MgCl_2$] = 10 mM; [EDTA] = 1 mM; [PDC-biotin] = 1 μM. Image size: 800 × 600 nm.

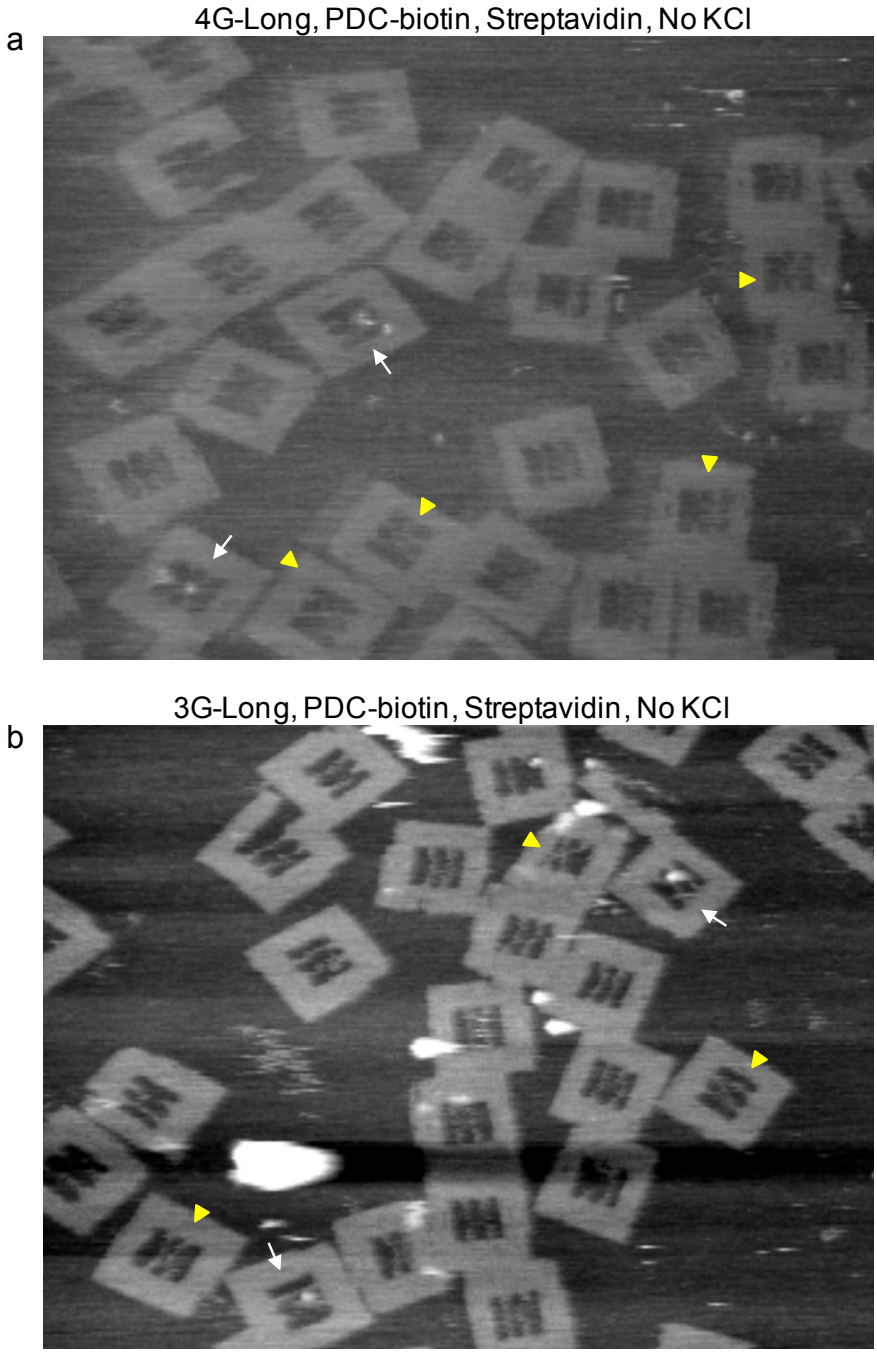


Fig. S3 Zoom-out images of the origami assembly with long-duplexes containing four (a) and three (b) contiguous guanines recorded in the presence of PDC-biotin and STV. Yellow arrow heads and white arrows indicate the X-shapes alone and STV-bound X-shapes, respectively. [Tris-HCl] = 20 mM, pH 7.6; [$MgCl_2$] = 10 mM; [EDTA] = 1 mM; [PDC-biotin] = 1 μM; [STV] = 0.2 μM. Image size: 800 × 600 nm.

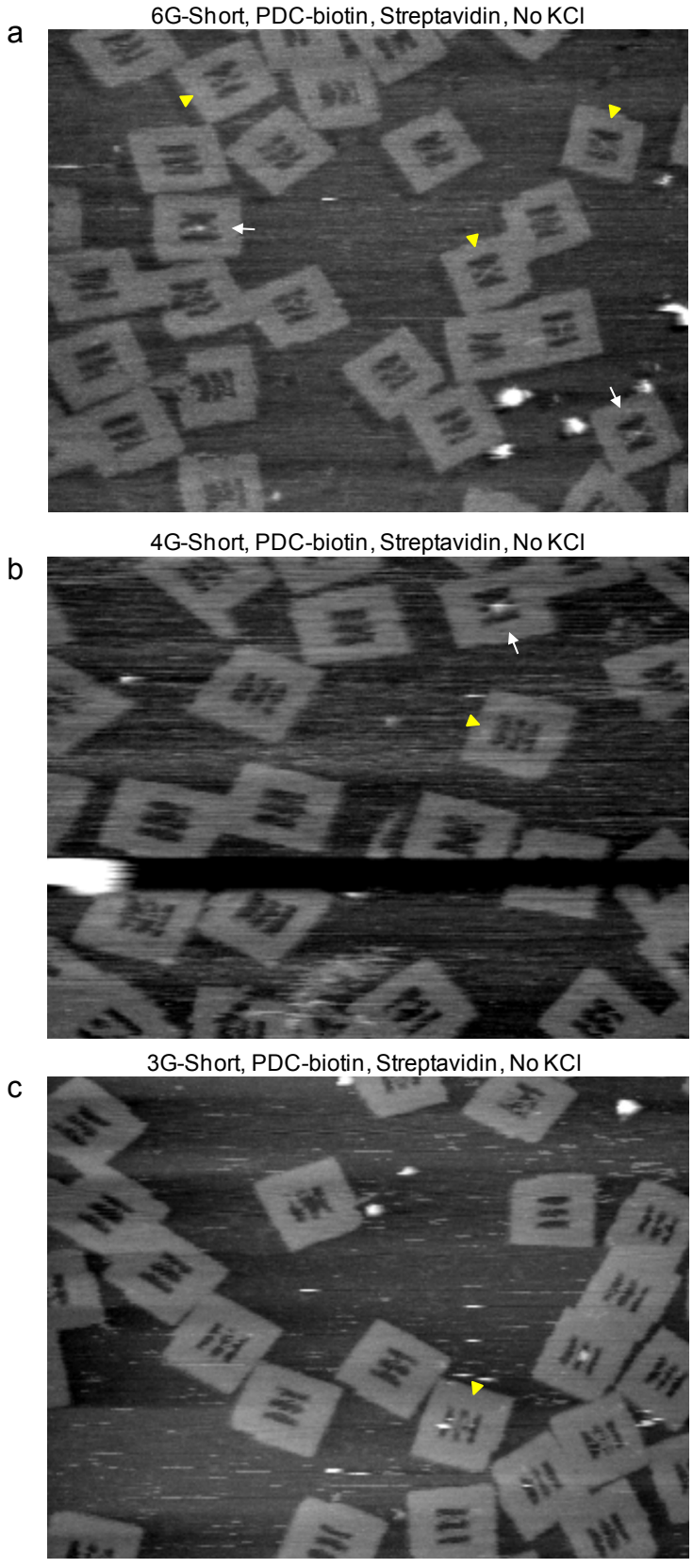


Fig. S4 Zoom-out images of the origami assembly with short-duplexes containing six (a), four (b) and three (c) contiguous Gs recorded in the presence of PDC-biotin and STV. Yellow arrow heads and white arrows indicate the X-shapes alone and STV-bound X-shapes, respectively. [Tris-HCl] = 20 mM, pH 7.6; [$MgCl_2$] = 10 mM; [EDTA] = 1 mM; [PDC-biotin] = 1 μM; [STV] = 0.2 μM. Image size: 800 × 600 nm (a and c), 696 × 522 nm (b).

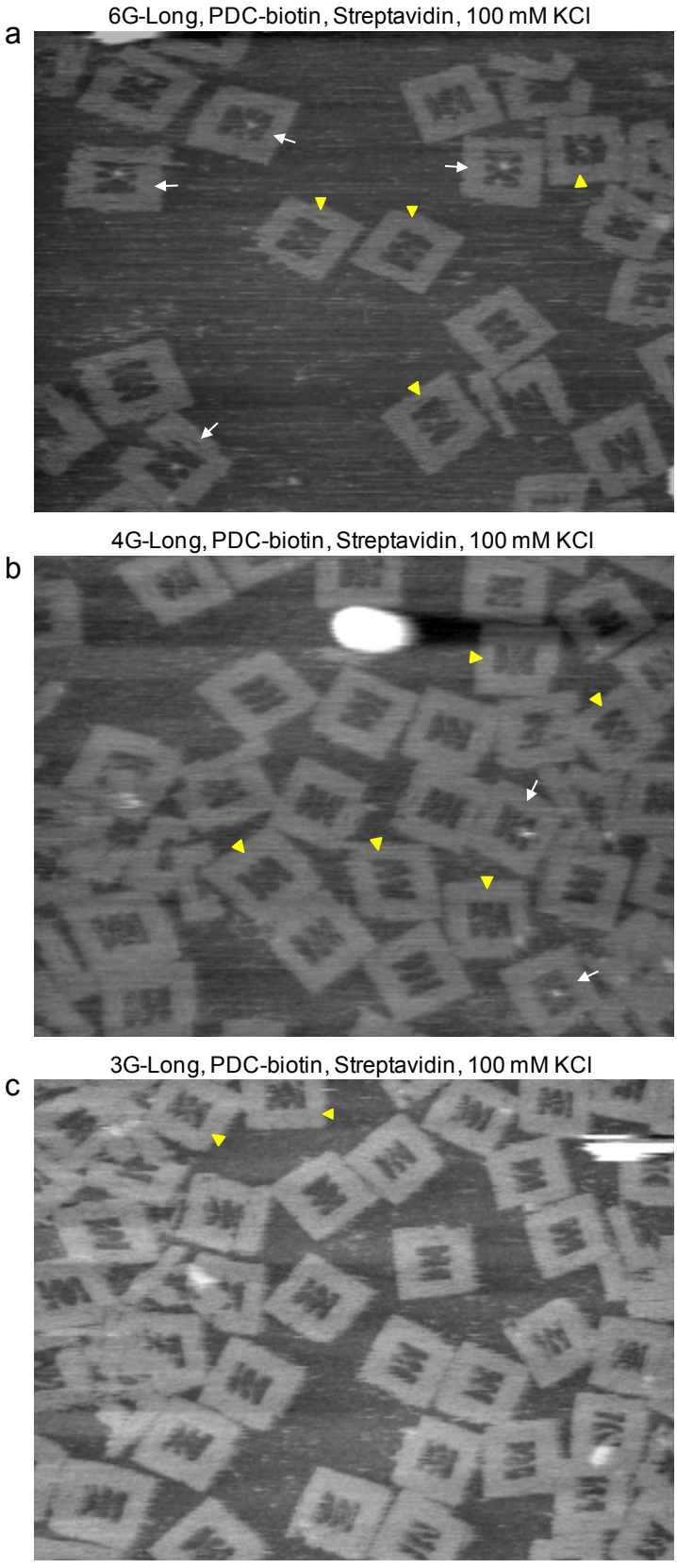


Fig. S5 Zoom-out images of the origami assembly with long-duplexes containing six (a), four (b) and three (c) contiguous Gs. Images recorded in the simultaneously presence of PDC-biotin, STV and KCl. Yellow arrow heads and white arrows denote the X-shapes alone and STV-bound X-shapes, respectively. [Tris-HCl] = 20 mM, pH 7.6; [$MgCl_2$] = 10 mM; [EDTA] = 1 mM; [PDC-biotin] = 1 μM; [STV] = 0.2 μM; [KCl] = 100 mM. Image size: 800 × 600 nm.

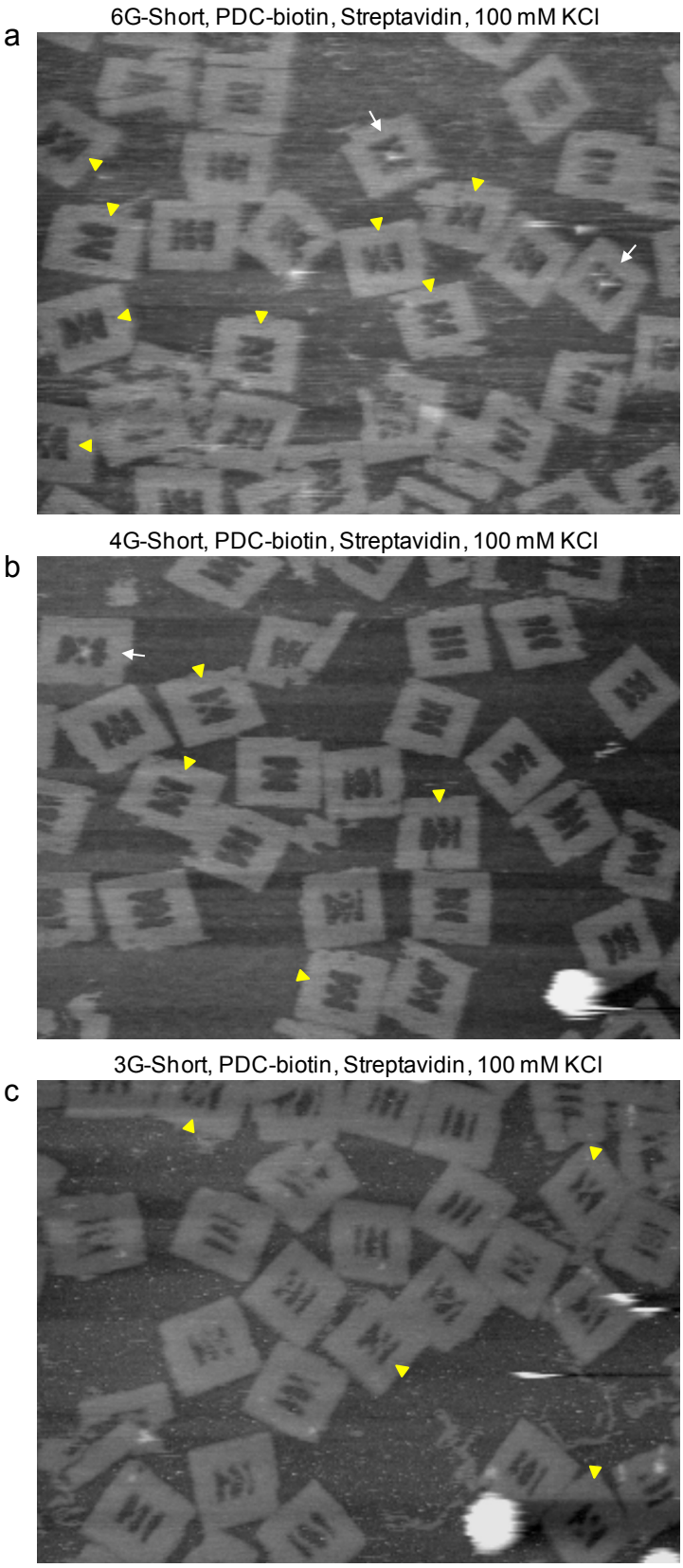


Fig. S6 Zoom-out images of the origami assembly with short-duplexes containing six (a), four (b) and three (c) contiguous Gs. Images recorded in the simultaneously presence of PDC-biotin, STV and KCl. Yellow arrow heads and white arrows denote the X-shapes alone and STV-bound X-shapes, respectively. [Tris-HCl] = 20 mM, pH 7.6; [$MgCl_2$] = 10 mM; [EDTA] = 1 mM; [PDC-biotin] = 1 μM; [STV] = 0.2 μM; [KCl] = 100 mM. Image size: 800 × 600 nm.

Top 1 : 5′-**CTGTAGCT CATCATGT** GGAGACTCTAGAGTGTTCCTGATGGCCGTA**(G)6**TCAAGGCGGTGGGTGCGCGTTGCTCCTCACT **GAACACCC TGAACAAA**-3′
Top 2 : 3′-CCTCTGAGATCTCACAAGGACTACCGGCAT**(T)6**AGTTCCGCCACCCACGCGCAACGAGGAGTGA-5′
Bottom 1 : 5′-**CGACAATA AACAACAT** GAGGAAGTGAGGAGCAACGCGCACCCACCGCCTTA**(T)6**TCACGGCCATCAGGAACACTCTAGAGTCTCCGCTCG **AGCAAACA AGAGAATC**-3′
Bottom 2 : 3′-CTCCTTCACTCCTCGTTGCGCGTGGGTGGCGGAAT**(T)6**AGTGCCGGTAGTCCTTGTGAGATCTCAGAGGCGAGC-5′

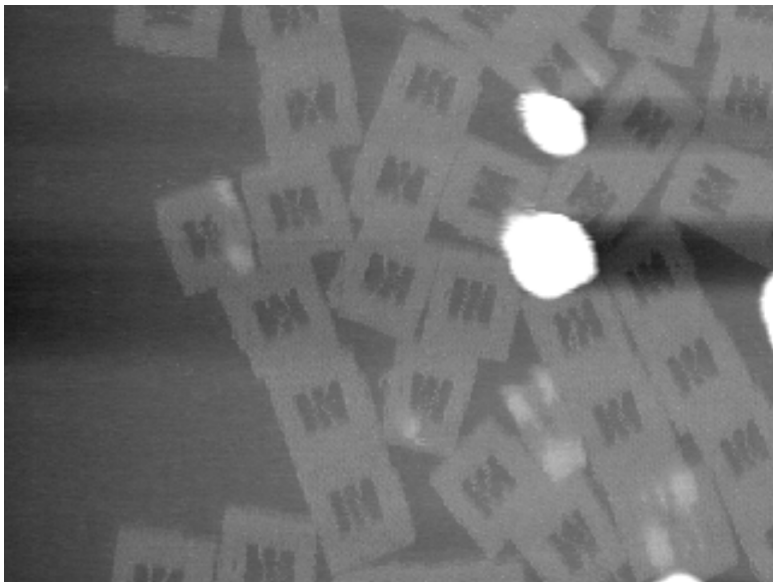


Fig. S7 Control experiment with six contiguous thymines and in the presence of PDC-biotin. Long-duplex system was used. Sequences used for this experiment are listed above the image. The absence of X-shape indicates the absence of the quadruplex structure. [Tris-HCl] = 20 mM, pH 7.6; [$MgCl_2$] = 10 mM; [EDTA] = 1 mM; [PDC–biotin] = 10 μM (in origami solution) or 1 μM (in observation buffer); [STV] = 0 μM; [KCl] = 0 mM. Image size: 800 × 600 nm.

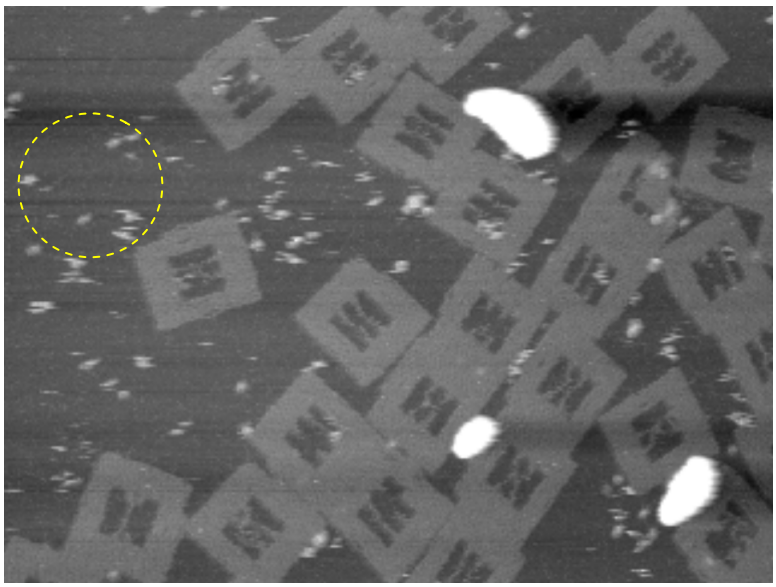


Fig. S8 AFM image of the origami assembly in the presence of STV alone. Long-duplex containing six contiguous Gs was used. The bright spots in the image (representative spots are marked by the dashed circle) represent the STV. In the absence of the ligand, addition of STV alone to the G-repeat sequence neither produced X-shape nor STV is strongly anchored on the DNA and/or X-shape, indicating that STV alone cannot induce the formation of the G-quadruplex structure. [Tris-HCl] = 20 mM, pH 7.6; [$MgCl_2$] = 10 mM; [EDTA] = 1 mM; [STV] = 2 μM; [PDC-biotin] = 0 μM; [KCl] = 0 mM. Image size: 800 × 600 nm.